Chlamydomonas reinhardtii data bases. The isomerization of the *all-trans*-retinal chromophore induces conformational changes of the protein that result in the opening of the channel pore to allow ion flow across the membrane. ChRs attract enormous attention because after expression in neuronal cells they can trigger action potentials upon blue light stimulation (450 nm). In 2007, we isolated two new ChRs from the fresh water algae *Volvox carterii* (VChR1 and VChR2). Notably VChR1 shows a red-shifted action spectrum peaking at 535 nm. Thus, VChR1 can be used to trigger action potentials in neurons by yellow light illumination [3]. Expression of ChR2 and VChR1 in different neuronal species enabled a distinct activation of the two cell types with blue and green light helping to understand neuronal circuits. But, a broader application of VChR1 was hampered because of its poor membrane localization and small currents in neurons. In this study we developed a well-expressing ChR with absorption characteristics similar to VChR1 by combining the N-terminal part of ChR1 and the C-terminal part of VChR1.

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APBSmem: A Tool for the Analysis of Membrane Protein Electrostatics

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Electrostatic forces orchestrate the folding of proteins, increase the binding of one protein to another and facilitate protein-DNA and protein-ligand binding. A popular means for computing the electrostatic properties of biological systems is to numerically solve the Poisson-Boltzmann (PB) equation, and there are several easy-to-use software packages available for carrying out these calculations on soluble proteins. We have developed a tool called APBSmem that performs these calculations in the presence of a membrane. Adaptive Poisson-Boltzmann Solver (APBS) is used as a back-end for solving the PB equation, and a graphical user interface (GUI) coordinates a set of routines that introduce the influence of the membrane, determine its placement and shape relative to the protein, and set the membrane potential. The software Jmol is embedded in the GUI to visualize the protein inserted in the membrane and the resulting electrostatic potential. We demonstrate the use of our software with three examples involving the calculation of the protein transfer free energy from water to membrane, solvation energy required to move an ion into a channel, and the gating charge of a molecular motion. We expect that the ease with which the GUI allows one to carry out these calculations will make this software a useful resource for experimenters and computational researchers alike. In particular, our built-in protocols should be appealing to researchers studying ion channel and transporter function.

3459-Pos Board B320

The Pivotal Twin-Histidine Element of the Escherichia Coli Ammonium Channel AmtB Functions as a Substrate Selectivity Filter

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Ammonium functions as both a primary nutrient and waste product and, thus, its transport across biological membranes is of fundamental importance. Because the uncharged form, NH_3, readily traverses phospholipid bilayers by simple diffusion the role of protein-catalyzed transport of the protonated species, NH_4^+, is unusually interesting. The Amt family of channels mediates the transport of NH_4^+ and is required for microbial growth when diffusion of NH_3 becomes limiting for nitrogen uptake. Whereas all other characterized channels facilitate downhill substrate movement, Amt proteins are active channels - hybrids between passive channels and active transporters - and concentrate NH_4^+ against a gradient. Amt family members function as homotrimers, with each monomeric unit carrying a pore for substrate conduction. Each pore is lined entirely with hydrophobic residues, save for a pair of conserved hydrogen-bonded histidines postulated to play a critical role in mediating NH_4^+ transport. We examined the impact that changes to this histidine pairing had on the function of one of the best-characterized members of the Amt family, the AmtB protein of *Escherichia coli*. Our initial analysis indicated that AmtB can accommodate, by either direct substitution or suppressor generation, acidic residues at one or both positions of the twin-histidine site while retaining good-to-excellent transport activity. Subsequent work shows that a number of mutant AmtB proteins carrying such alterations leak K^+ ions and that this leakage is energetically costly. These findings lead us to conclude that whereas the twin-histidine element is not required to conduct NH_4^+ it serves as a filter to prevent AmtB-mediated K^+ transport.

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Mapping Water Density to Design New Blockers Against a Viral Proton Channel

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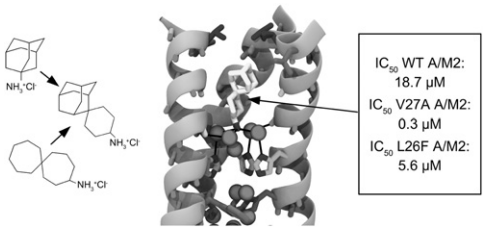
The influenza A virus utilizes a membrane-embedded proton channel, M2. Its functions are to acidify the viral interior and trigger the uncoating of its RNA, equilibrate the pH across the Golgi during replication, and allow a fully formed virus to bud from the host cell. One of the two classes of approved anti-influenza drugs contains amphiphilic molecules such as amantadine, that prevent extraviral H+ and water from accessing the pore of M2. Drug-resistant mutations (now pervasive through most of the flu strains) feature a more hydrated pore that destabilizes amantadine binding. We have used molecular dynamics simulations over extended times, reconstructed accurate water density maps, and identified several metastable positions of amantadine in the wild-type protein and its mutants. Using these data we designed new amine-based inhibitors, that fully suppress H+ conduction and viral replication in drug resistant strains.



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Effects of Substance P on Excitability of Dorsal Root Ganglion Neurons

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Some primary nociceptor neurons produce and release substance P (SP), a peptide neurotransmitter with well-described effects on second-order sensory neurons. However, the effects of SP on primary sensory neurons are less clear. We tested the hypothesis that SP acts on an autoreceptor by examining the pharmacological profile of rat DRG neurons sensitive to SP. Whole-cell patch clamp was used to measure the response of 89 cells to brief applications of pH 7 (27 positive cells), pH 6 (37 positive cells), capsaicin (22 positive cells), and ATP (15 P2X3 type positive cells). Sensitivity to SP was determined by an increase in cell excitability measured as the number of action potentials at the threshold and the slope of the stimulus-response curve (16 positive cells). There was also a decrease in excitability in 8 cells. Among the cells responding to SP by increasing excitability, the frequencies of sensitivity to pH 7 (67%) and pH 6 (88%) were higher than in non-responding cells (24% and 32 % respectively, $p<0.01$). The frequencies of sensitivity to capsaicin had a tendency to be higher in the SP responding cells (38% versus 22%, $p=0.33$). P2X3 type ATP currents were present in 15 of 73 (21%) SP non-responding cells, while none of the 16 SP positive cells presented this current ($p=0.1$). We conclude that the majority of SP sensitive neurons exhibit a pharmacological profile typical of nociceptors, although P2X3 currents were not present in these cells.

3462-Pos Board B323

Ion Channel Proteins that Spontaneously Insert into Lipid Bilayer Membranes: An Impedance Spectroscopy Study Employing Tethered Membranes

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The Chloride Intracellular Ion Channel (CLIC) protein family, the Annexins, the bacterial toxins Hemolysin (AH), Streptolysin, Perfringolysin Listeriolysin, Pneumonlysin, Ivanolysin and Colicins all possess unusual mechanisms for inserting into cellular and/or host membranes. They are representatives of proteins that spontaneously insert into membranes, by-passing the route for the incorporation of most integral membrane proteins. For example, the soluble form of the 240-amino acid polypeptide CLIC1 is known to exist in at least two conformations due to a large rearrangement of its amino terminus under the influence of oxidation. Oxidation promotes its binding to and insertion

into lipid bilayers, forming a chloride selective ion channel. Spontaneous membrane insertion also occurs for the 293-amino acid polypeptide, AH which is secreted as a water-soluble monomer, and when in contact with a membrane inserts and forms heptameric pores. The 36-amino acid peptide (pHLIP) which is a truncated sequence of the C-helix of the integral membrane protein bacteriorhodopsin also inserts into lipid bilayers at low pH. In the case of the 316-amino acid polypeptide Annexin B12, reversible insertion into membranes can also occur at acidic pH. In the present study, we report the functional ion channel activity of a number of spontaneously inserting proteins into Tethered Membranes via a novel impedance spectroscopy assay. We report on conditions that promote insertion and determine the ease of reversible dissociation of protein from the membrane. Also reported will be the kinetics of both the insertion and elimination of the protein based on the functional conductance changes of the membrane.

3463-Pos Board B324

Single Channel and Ensemble Measurements of CLIC1 in Lipid Bilayers

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Abstract

Chloride intracellular channel 1 (CLIC1) is an ion channel protein which has been hypothesized to play a fundamental role in neurodegenerative diseases like Alzheimer's disease (AD) [1]. To date, single-channel characteristics of this protein have been obtained by reconstituting it in lipid bilayers and whole cell patch clamp measurements of overexpressing cells. CLIC1 protein can exist in both soluble and integral membrane form[2] and localizes on the plasma membrane and intracellular organelles. Obtaining ensemble measurements of CLIC1 by whole cell patch clamp is very difficult, due to its subcellular localization and its solubility in cytoplasm [3]. We report single channel and ensemble measurements of reconstituted CLIC1 in artificial lipid bilayers formed using sessile droplets, a measurement platform amenable to parallel and automated ion channel studies.[4] We also measured dose dependent inhibition of CLIC1 multi-channel currents by a known blocker, IAA94. This work may be applicable to measurement and screening of other intracellular ion channels as well.

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3464-Pos Board B325

Electrokinetically Altered Normal Saline Modulates Ion Channel Activity

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Normal saline (0.9% NaCl) was subjected to Taylor-Couette-Poiseuille (TCP) flow in the presence of oxygen in a mixing device that facilitates controlled turbulence and cavitation events. The resultant solution, RNS60, is proposed to contain charge-stabilized nanostructures consisting of a nanobubble core surrounded by an electrical double-layer at the liquid/gas interface. Through various methods (ICP, TOF mass spectroscopy, and UV-Vis, NMR, Raman spectroscopy) we find that RNS60 has no detectable contaminant species, and that the fluid is chemically equivalent to normal saline. Nanoparticle tracking analysis provides evidence for the presence of nanoscale structures in RNS60 bulk fluid, and tapping-mode AFM observations reveal differences in the nanobubbles formed on hydrophobic surfaces.

Using whole-cell electrophysiology, we have detected bioactive interactions of RNS60 with the cell membrane. Transient receptor potential type V1 (TRPV1) current is strongly inhibited by physiological saline containing RNS60. Prolonged activation by capsaicin is significantly reduced (−85.9 +/− 7.0 %), while perfusion switch to RNS60 solution during acute application of capsaicin also shows rapid inhibition (−42.5 +/−14.7%). Further, with cys-loop protein family member 5HT3A, potentiation of serotonin-evoked current is observed (101.9 +/− 24.2%). These results suggest that a stable, chemically unaltered saline solution is able to interact strongly on the biological membrane to modulate activity of specific ion channels.

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Analysis of Multichannel Signals using a Channel Simulator

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Measurements of ion channel activities that generate multichannel events are important to understand, but difficult to analyze. In many cases the multichannel events are discarded instead of used in the analysis. The availability of a channel signal simulator offers an excellent opportunity to develop and test statistical models for analysis of multichannel signals. We have generated single channel traces for various open probabilities and then digitally superimposed these signals to obtain multichannel events. We then applied our analysis to these multichannel traces to calculate single channel parameters such as the average ON and OFF times and their second moments. In addition to allowing testing of statistical calculations, simulated channel data has pedagogical value when used as tutorials in statistical analysis, especially with regard to binomial distributions.

Voltage-gated K Channels: Gating III

3466-Pos Board B327

Barium Ions Selectively Activate BK Channels through the Ca^{2+}-Bowl Site

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The BK-type K^+ channel is regulated by two distinct high affinity divalent cation sensitive sites on each Slo1 α subunit. One site, termed the Ca^{2+} bowl, is associated with the RCK2 domain of each α subunit, while acidic residues in the RCK1 domain have been linked to a separate Ca^{2+}-ligation site. Although both sites are activated by Ca^{2+} and Sr^{2+}, Cd^{2+} has been shown to favor activation via the regulatory site in RCK1. Here we examine the ability of Ba^{2+} to activate BK channels. Although Ba^{2+} is well-known as a potent blocker of K^+ channels, we show that, before the onset of channel block by Ba^{2+}, step increases in Ba^{2+} to low μM concentrations result in modest activation of BK channels. nPo measurements at negative potentials, where blockade by Ba^{2+} is alleviated, confirms that the activating effect of 10 μM Ba^{2+} on wild type BK channels is comparable to that of ~4 μM Ca^{2+}. By examining the ability of Ba^{2+} to activate BK channels either through the mutationally defined RCK1 site or the Ca^{2+} bowl site, we further show that this Ba^{2+}-dependent activation is mediated almost entirely by the Ca^{2+} bowl site but not the RCK1 site. These results have two implications. First, although functional activation by Ba^{2+} will rarely be discerned, Ba^{2+} may induce conformational effects on the BK channel independent of block. Second, the results provide new information regarding divalent cation selectivity of the two BK high affinity binding sites. Whereas both sites are activated by Ca^{2+} and Sr^{2+}, RCK1 is selective for Cd^{2+}, while the Ca^{2+} bowl is selective for Ba^{2+}. These results add further support to the view that two functionally distinct high affinity divalent cation sites with different selectivity on ionic radius regulate BK function.

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The BK Channel Opener Phloretin Influences Voltage- and Calcium-Dependent Gating

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Large-conductance (BK), voltage- and Ca^{2+}-activated potassium channels are considered an attractive therapeutic target. In order to facilitate drug development and the identification of clinically useful compounds, we analyzed the mechanism of action of BK openers in the context of an allosteric gating scheme. The focus of the current study is the BK opener phloretin. Openers may 1) enhance opening of the gate, 2) enhance voltage- and/or Ca^{2+}-sensor activation, or 3) perturb coupling of voltage- and/or Ca^{2+}-sensors to the gate. To distinguish among these possibilities, unitary and macroscopic currents were recorded from heterologously-expressed BK channels composed of human α subunits (hSlo1) in different Ca^{2+} concentrations over a wide range of voltage and P_o. The predominant effect of phloretin (100 μM) was to increase P_o with greater efficacy at negative voltages (~100-fold increase in 0 Ca^{2+}) than at positive voltages, an effect consistent with actions on the gate and/or its coupling to voltage-sensors. When tested on mSlo R210C, a mutant with constitutively activated voltage-sensors, phloretin produced a five-fold increase in nP_o. This increase was significantly less than that observed for wild-type channels, further supporting phloretin's effects on